A STUDY OF STREPTOCOCCUS IMMUNIZATION.*

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A STUDY OF THE IMMUNIZING EFFECTS AGAINST HOMOLOGOUS ORGANISMS OF INJECTIONS IN RABBITS OF STREPTOCOCCI KILLED BY HEAT AND KILLED BY CHEMICALLY INDIFFERENT AGENTS.

WHILE engaged in the study of streptococcus infections and immunity our attention was directed to some results obtained in connection with tubercle, typhoid, and glanders bacilli by Levy, Blumenthal, and Marxer.[1] They obtained a considerable degree of immunity in animals by the injection of bacteria which had been killed by chemically indifferent agents, such as sugars, glycerin, and urea. Virulent bacteria, devitalized by suspension in glycerin or strong solutions of sugars and urea, could be injected in relatively large quantities with little or no apparent effect upon the animal, but the injections were followed by a marked immunity for the corresponding living bacteria.

Having employed injections of streptococci killed by heat in the treatment of cases of streptococcus infection (see p. 585) without satisfactory results, we undertook to determine in animals the effects of the injection of virulent streptococci, killed by suspensions in a strong solution of galactose, and to compare the results thus obtained with those observed when streptococci killed by heat were injected. Rabbits were used in all the experiments, and the streptococcus cultures employed were made virulent for rabbits by passages through this animal. The streptococci were grown upon the surfaces of blood-agar slants in ordinary test-tubes. After 24 hours at 35° C. the fluid of condensation was removed and the bacteria upon the surface suspended in sterile, 25 per cent solution of galactose. Two c.c. of the solution was employed for each slant. The suspension was kept in the incubator for 48 or 72 hours, being shaken several times in the interval. The suspension was then distributed in small test-tubes,

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[1] *Centralbl. f. Bakt.*, 1906, 42, p. 265.

each tube containing one-half the growth from a blood-agar slant. After thorough centrifugalization, the clear supernatent fluid was carefully pipitted off. The contents of the tubes were then rapidly desiccated in vacuum over calcium chloride at room temperature, then sealed and kept in the refrigerator. The sterility of the suspension was always determined by cultures. Usually all the cocci were dead in 24 hours. Only one streptococcus with which we have worked failed to be killed in 48 hours. The suspension of this latter organism became sterile only after 72 hours. When the killed organisms were to be injected, they were suspended in 2 or 3 c.c. sterile salt solution.

Numerous estimates of the numbers of cocci in such growths have shown that the average growth on a slant of blood agar after 24 hours comprises approximately 1,000,000,000 cocci.

When the streptococci were killed by heat, they were washed from the blood agar with 0.85 per cent sodium chloride solution, and the suspension heated to 60° for 30 minutes and kept in the ice-box.

Effects of the injection into rabbits of streptococci killed by galactose solution.—Rabbits were given one or more subcutaneous injections of streptococci killed by galactose, and examined as to the effects upon the opsonic index and as to subsequent resistance to living streptococci of the same strain as that from which the killed cocci were obtained. In no case did the injections produce any appreciable effect upon the animal other than a slight induration at the point of injection. This induration was not larger than a pea and was not accompanied by any inflammatory phenomena.

After galactose killed streptococci were injected into rabbits the opsonic index was found to follow a more or less regular course. A negative phase or fall in the index was inconstant and not usually pronounced. The fall was usually more marked after the primary than after subsequent injections. The index was usually highest on the second or third and fourth or fifth days after the injection. The character of the curve was similar with doses of varying size, but the larger doses usually produced higher indices.

When rabbits are injected subcutaneously with galactose killed streptococci, more or less immunity to the corresponding living streptococcus develops. After a single injection five to seven days

are required for this to appear. The protection afforded by two injections is greater than that following a single one. The accompanying tables will show the results obtained in representative instances:

TABLE 1.

Rabbit N, 1,220 grms.	Aug. 12, 1907. 1,000,000,000 galactose killed Streptococcus No. 3 subcutaneously	Aug. 17, 1907 2 blood-agar slants growth of Streptococcus No. 3 into peritoneal cavity	Died 4 days after inoculation
Rabbit O, 1,260 grms.	Aug. 13, 1907–do	do	Died 48 hrs. after inoculation
Rabbit P, 1,390 grms.	Aug. 14, 1907–do	do	Died 18 hrs. after inoculation
Rabbit Q, 1,120 grms.	Aug. 15, 1907–do	do	Died 22 hrs. after inoculation
Rabbit R, 1,420 grms.	Aug. 16, 1907–do	do	Died 48 hrs. after inoculation
Rabbit S, 1,410 grms.	Control	do	Died 18 hrs. after inoculation

In this experiment the killed streptococci were those from a single suspension equally divided. The living culture injected on August 17 consisted of the growth on 12 blood-agar slants, suspended in broth and divided into six equal parts.

TABLE 2.

Rabbit J, 1,600 grms.	July 30, 1907 500,000,000 galactose killed Streptococcus No. 3 subcutaneously Aug. 2, 1907 500,000,000 galactose killed Streptococcus No. 3 subcutaneously	Aug. 8, 1907 1 blood-agar slant growth of Streptococcus No. 3 into peritoneal cavity	Well a month after inoculation
Rabbit K, 1,500 grms.	July 30, 1907 500,000,000 galactose killed Streptococcus No. 3 subcutaneously Aug. 2, 1907 500,000,000 galactose killed Streptococcus No. 3 subcutaneously	do	Well a month after inoculation
Rabbit M, 1,710 grms.	Control	do	Died 18 hours after inoculation

In this experiment the killed streptococci were those from equal parts of a single suspension. The living cultures injected consisted of the growth from three slants of blood agar, suspended in broth, and divided into three equal portions.

TABLE 3.

Rabbit 1, 1,340 grms.	Aug. 29, 1908 1,000,000,000 galactose killed Streptococcus A. subcutaneously Sept. 3, 1908 1,000,000,000 galactose killed Streptococcus A. subcutaneously	Sept. 9, 1908 5 c.c. 24 hours broth culture Streptococcus A. intraperitoneally	Sept. 10 Slightly sick; Sept. 11 quite well; well a month later
Rabbit 2, 1,250 grms.	Control	Sept. 9, 1908 5 c.c. 24 hours broth culture Streptococcus A. intraperitoneally	Died 15 hours after inoculation
Rabbit 3, 1,540 grms.	Oct. 5, 1908 500,000,000 galactose killed Streptococcus A. subcutaneously	Oct. 15, 1908 0.2 c.c. 24 hrs. broth culture Streptococcus A. subcutaneously in ear	Oct. 16: Ear red and a little swollen Oct. 18: Ear much swollen —acts sick Oct. 19: Worse Oct. 20: Slightly better Oct. 21: Ear better. General condition much improved Oct. 22: Died
Rabbit 4, 1,765 grms.	Control	Oct. 15, 1908–do	Oct. 16: Ear red and a little swollen Oct. 18: Died

Comparison of the effects of the injection into rabbits of streptococci killed by galactose solution and by heat.—In testing the relative effect in rabbits of streptococci which were killed by suspension in 25 per cent galactose solution and by heat, individual animals were injected subcutaneously with equal amounts of the same culture of streptococci killed in each of the two ways. Those killed by heat were suspended in 0.85 per cent sodium chloride solution and heated to 60° for 30 minutes. Those killed in galactose solution were treated as already indicated. In every case the suspensions were sterile. After sufficient

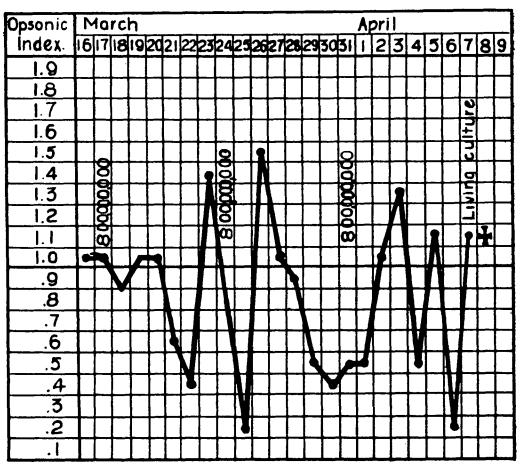

CHART 1.—Streptococco-opsonic index in Rabbit 1, Table 4, with approximate number of bacteria injected.

time had elapsed to allow some degree of immunity to have developed, the animals of the series and a control animal were injected with equal amounts of a culture of the same streptococcus as that from which the killed organisms were obtained. Determinations of the opsonic index were also made in the rabbits during the experiment.

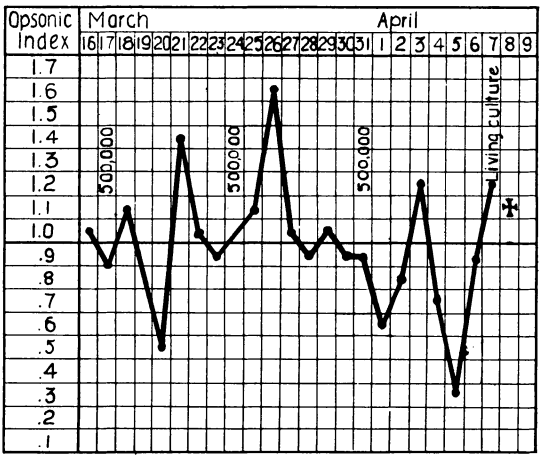

CHART 2.—Streptococco-opsonic index in Rabbit 2, Table 4, with approximate number of bacteria injected.

The following tables show the results obtained in three experiments. Accompanying these tables are charts of the opsonic index in the individual animals of the series:

TABLE 4.

Rabbit 1, 1,040 grms.	Mar. 17, 1908 800,000,000 Streptococcus J. K. 13, killed by heat, subcutaneously Mar. 24, 1908–do Mar. 31, 1908–do	April 7, 1908 8 c.c. 20 hours broth culture Streptococcus J. K. 13, intraperitoneally	Died in less than 24 hrs. after inoculation
Rabbit 2, 1,160 grms.	Mar. 17, 1908 500,000 Streptococcus J. K. 13, killed by heat subcutaneously Mar. 24, 1908–do Mar. 31, 1908–do	do	Died in less than 24 hrs. after inoculation
Rabbit 3, 940 grms.	Mar. 17, 1908 800,000,000 galactose killed Streptococcus J. K. 13, subcutaneously Mar. 24, 1908–do Mar. 31, 1908–do	do	Died between 2 and 3 days after inoculation
Rabbit 4, 1,200 grms.	Mar. 17, 1908 500,000 galactose killed Streptococcus J. K. 13, subcutaneously Mar. 24, 1908–do Mar. 31, 1908–do	do	Died between 1 and 2 days after inoculation

Unfortunately the control rabbit for this experiment received at least part of its injection of the living cultures in the abdominal wall

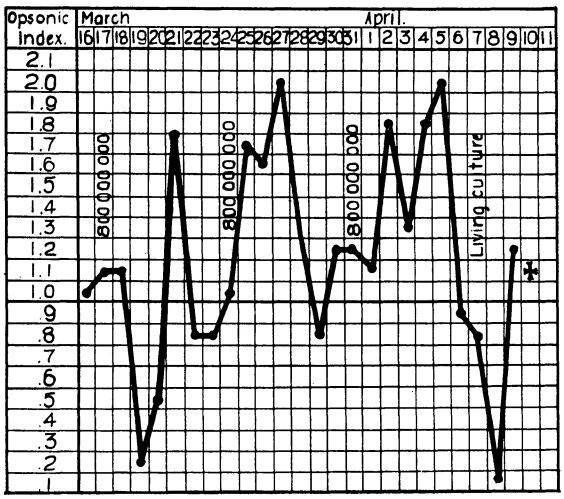

CHART 3.—Streptococco-opsonic index in Rabbit 3, Table 4, with approximate number of bacteria injected.

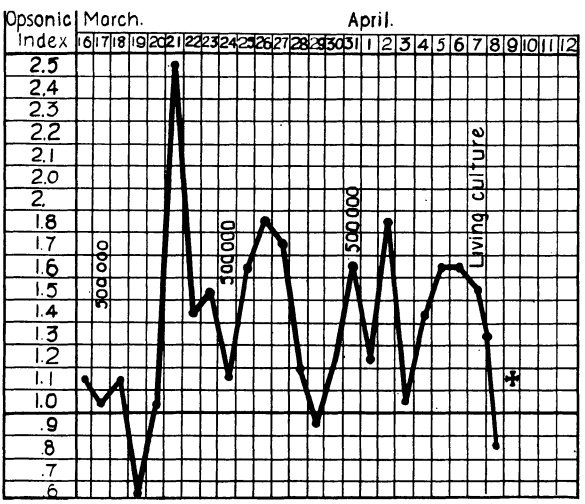

CHART 4.—Streptococco-opsonic index in Rabbit 4, Table 4, with approximate number of bacteria injected.

where a marked local swelling developed, but the animal lived.

TABLE 5.

Rabbit 1, 2,300 grms.	Sept, 22, 1908 1,000,000,000 heat killed Streptococcus A. subcutaneously Sept. 26, 1908–do	Oct, 2, 1908 5 c.c. 24 hrs. broth culture Streptococcus A. intraperitoneally	Very sick 1½ hrs. after inoculation Died in 8 hrs. after inoculation
Rabbit 2, 2,190 grms.	Sept. 22, 1908 1,000,000,000 galactose killed Streptococcus A. subcutaneously Sept. 26, 1908–do	Oct. 2, 1908–do	Not sick 1½ hrs. after inoculation Died 14 hrs. after inoculation
Rabbit 3, 2,100 grms.	Control	Oct. 2, 1908–do	Very sick 1½ hrs. after inoculation Died in 8 hrs. after inoculation

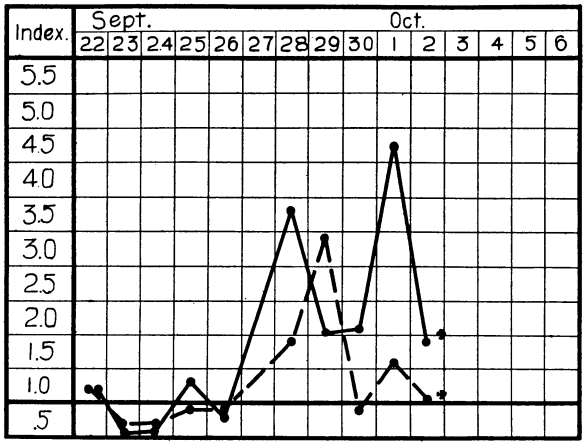

CHART 5.—Solid line, streptococco-opsonic index in Rabbit 2; broken line, streptococco-opsonic index in Rabbit 1, Table 5.

The dose of living streptococcus in this experiment was massive. One c.c. of the same culture killed a 2,000-gram rabbit in two days. If a somewhat smaller dose had been used, it is likely that more difference between the resistance of the animals would have been observed. The experiment is introduced as the opsonic curves are very typical.

TABLE 6.

Rabbit 1, 1,450 grms.	Oct. 5, 1908 500,000,000 galactose killed Streptococcus A. subcutaneously Oct. 9, 1908–do	Oct. 15, 1908 3 c.c. 24 hrs. broth culture Streptococcus A. intraperitoneally	Never sick and was well a month later
Rabbit 2. 1,400 grms.	Oct. 5, 1908 500.000,000 heat killed Streptococcus A. subcutaneously Oct. 9, 1908–do	Oct. 15, 1908–do	Died 12 hrs. after inoculation
Rabbit 3. 1,350 grms.	Control	Oct. 15, 1908–do	Died 36 hrs. after inoculation

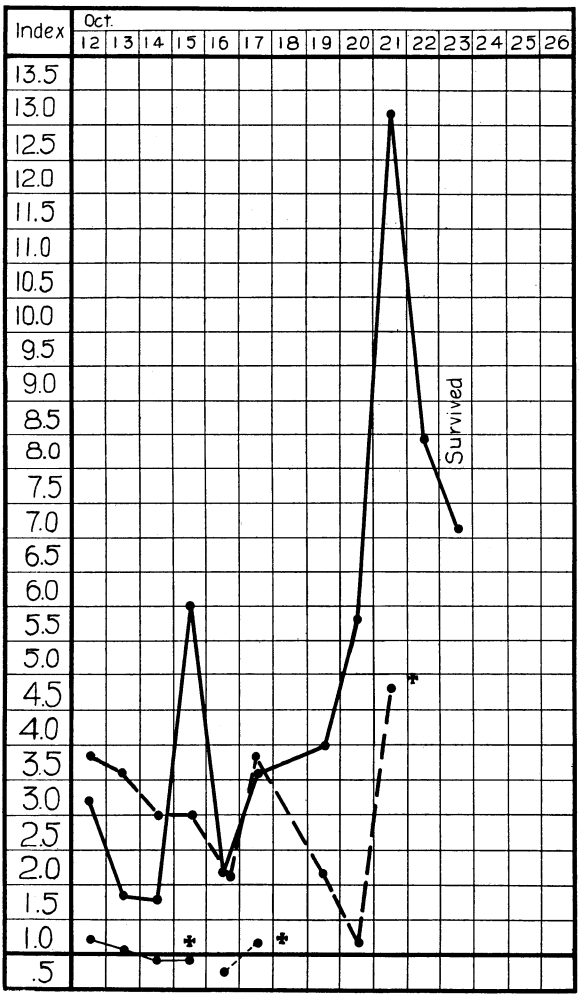

CHART 6.—Heavy solid line, streptococco-opsonic index in Rabbit 1, Table 6; lighter solid line, streptococco-opsonic index in Rabbit 2, Table 6; heavy broken line, streptococco-opsonic index in Rabbit 3, Table 3; lighter broken line, streptococco-opsonic index in Rabbit 4, Table 3.

From a study of the observations related above certain conclusions seem apparent. The subcutaneous injections of streptococci killed in galactose solution are followed by very definite phenomena. The serum of rabbits after such injections contain considerable amounts of opsonin which render the corresponding virulent streptococci susceptible to phagocytosis. Hand in hand with this rise of the opsonin, there develops in the rabbit a considerable degree of immunity to the living virulent streptococcus. The immunity thus acquired is sufficient to protect the experimental animal against such doses as kill acutely normal animals. The protection may be complete with a given dose, or may delay and modify the infection.

In marked contrast to this are the effects noticed after the injection into rabbits of streptococci killed by heat. Such rabbits do not produce any pronounced amount of opsonin for streptococci, and when injected with living cultures seem to have less resistance than normal rabbits.

EFFECTS OF THE INJECTION INTO MAN OF STREPTOCOCCI KILLED BY GALACTOSE SOLUTION.

Having found that streptococci killed by immersion in strong solution of galactose were without toxic effect in rabbits, it was decided to determine the results of such injections in man. Streptococci to the number of 500,000,000 killed in this way were injected in a healthy person beneath the skin. Following the injection there was no local reaction observed. Daily estimates of the opsonic index for the streptococcus, pneumococcus, and staphylococcus (*aureus*) were made, and the findings are shown in the accompanying chart (Chart 7). There is seen to be a double rise in the opsonic index for the streptococcus with no initial negative phase. The index for the pneumococcus and staphylococcus remained within normal limits.

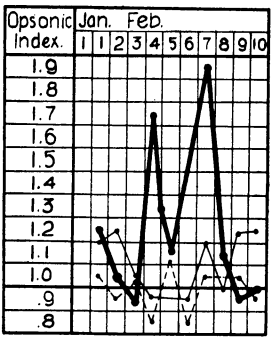

CHART 7.—Opsonic indices in a healthy individual after a single injection of 500,000,000 streptococci killed in galactose solution; heavy solid line for streptococcus, lighter solid line for staphylococcus, broken line for pneumococcus.

INJECTIONS OF HOMOLOGOUS STREPTOCOCCI KILLED BY GALACTOSE SOLUTION IN THE TREATMENT OF STREPTOCOCCUS INFECTIONS IN MAN.

Encouraged by the results obtained in animals, injections of streptococci killed by galactose solution were employed in the treatment of patients with chronic streptococcus infections. The results have been so gratifying that the history of two such cases in which the opsonic index curve is complete are here reported. In each case the streptococcus employed was obtained from the patient to be treated, and the material for injection was prepared in the same manner as that used in the experiments on rabbits.

Case I.—Boy, age 5 years. Post-scarlatinal suppurative otitis media and mastoiditis.

June 5, 1907: Eleven days previously the boy had been discharged from Cook County Hospital after recovery from a moderately severe attack of scarlatina. Six days ago the cervical glands began to swell, and later an abscess in the neck opened spontaneously. At this time a discharge from the right ear made its appearance. Today on admission to the hospital there is a purulent discharge from the right ear, the cervical glands on the right side beneath the jaw are swollen and tender. On the left side of the neck there is a swelling as large as a walnut from which pus exudes through a narrow sinus.

June 11: Signs of mastoid involvement on the right side.

June 14: The mastoid opened and much purulent fluid evacuated; profuse purulent discharge has continued from the right ear.

June 24: Profuse, foul, discharge from right ear and mastoid wound.

July 9: Profuse discharge continues from both sources. The temperature has been irregular, running from 99° to 104° F. The pulse has become irregular and the general condition of the patient is bad.

July 17: 250,000,000 of galactose killed streptococci injected subcutaneously. The streptococcus was obtained from the pus from the mastoid wound, which yielded almost a pure growth of typical hemolyzing colonies in blood-agar plates.

Following the injection the temperature became normal in two days, and never again rose above 99.6° F. The general condition of the

patient also began to improve and the improvement was progressive. Injections were made on July 22 of 500,000,000; on July 29 of 250,000,000; and on Aug. 9 of 250,000,000 galactose killed streptococci. The accompanying chart (Chart 8) shows the course of the case with opsonic-index determinations. In this case the tendency had been to chronicity and the patient was constantly growing worse until the injections were begun. After the injections were begun the patient immediately began to improve generally, and the local condition in the ear and mastoid also became better. The improvement

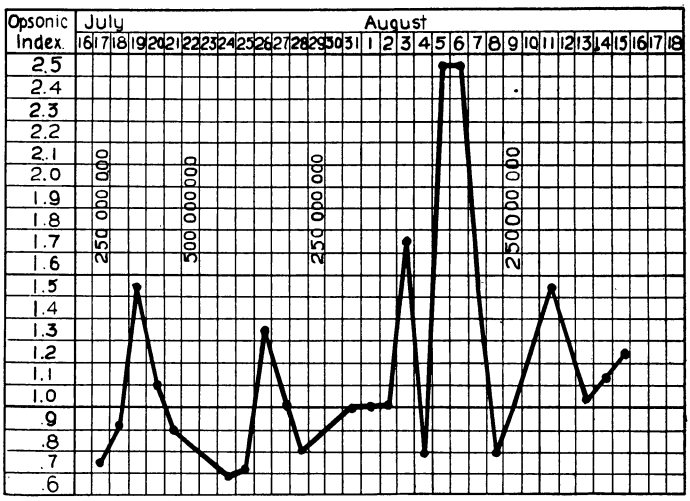

CHART 8.—Streptococco-opsonic index in Case I, p. 598, with approximate number of bacteria injected.

was especially rapid and marked when the index was highest on August 4 to 8.

Case II.—Boy, age 6 years. Chronic erysipelas with recurring acute attacks.

May 27, 1907: Upon this date the patient was admitted to the Home for Destitute Crippled Children because of a tuberculous spondylitis with associated discharging sinuses in the thigh. At this time there was a general swelling of the soft tissues of the face, being most marked about the eyes and nose. As to the previous history it was only possible to learn that the condition in the face had existed for a considerable time.

June 6: The boy had a chill with fever. A red spot appeared on one side of the nose, and the redness with swelling rapidly spread over the face. By June 10, when he was transferred to the contagious wards of Cook County Hospital, his condition was as follows: The face was enormously swollen and edematous; the eyes closed by the swelling. The skin was indurated, hard, reddish. The redness and swelling were sharply outlined, and included the external ears and forehead. There was considerable epithora. The conjunctivae were injected. The temperature was 104° F. per rectum.

For a week the acute symptoms continued with an irregular temperature ranging from 102 to 105° F., and a pulse from 100 to 130. There was a profuse sero-purulent nasal discharge and double suppurative otitis media developed. The patient was very irritable and at times actively delirious.

From June 18 to July 19 the temperature was normal except for an occasional rise to 99 or 100° F. During the sickness repeated examinations failed to disclose albumen in the urine. Profuse purulent discharge from the sinuses in the thigh was present during all this period.

July 19 he was discharged from the hospital and returned to the Home for Crippled Children. At this time all acute symptoms were absent, but there was still present considerable swelling of the face. From July 19, 1907, to January 17, 1908, when the first injection of dead streptococci was given, his condition was constantly as follows: The skin of the face, including the ears and forehead, was much swollen, firm, waxy white. The thickening was most marked over the bridge of the nose, the upper lip, and lower eyelids. The swelling of the eyelids was often so severe as to prevent the opening of the eyes. There was constant epiphora from occlusion of the nasal duct. The conjunctivae were reddened and one cornea presented an opaque area. The nasal mucous membrane was much swollen; the nasal passages admitted only a small applicator. From the nose there was a constant sero-purulent discharge giving rise to excoriation of the skin of the upper lip. Cultures from the nasal secretions gave large numbers of streptococci, which produced hemolyzing colonies in blood-agar plates. There were few other bacteria present. The sinus in the thigh almost closed at times, but with

the recurrences in the face the discharge always became more profuse. Cultures from the sinus discharge yielded large numbers of hemolyzing streptococcus colonies, almost in purity.

The accompanying photograph was taken at a time when the face was at its best, and shows the general swelling of the tissues.

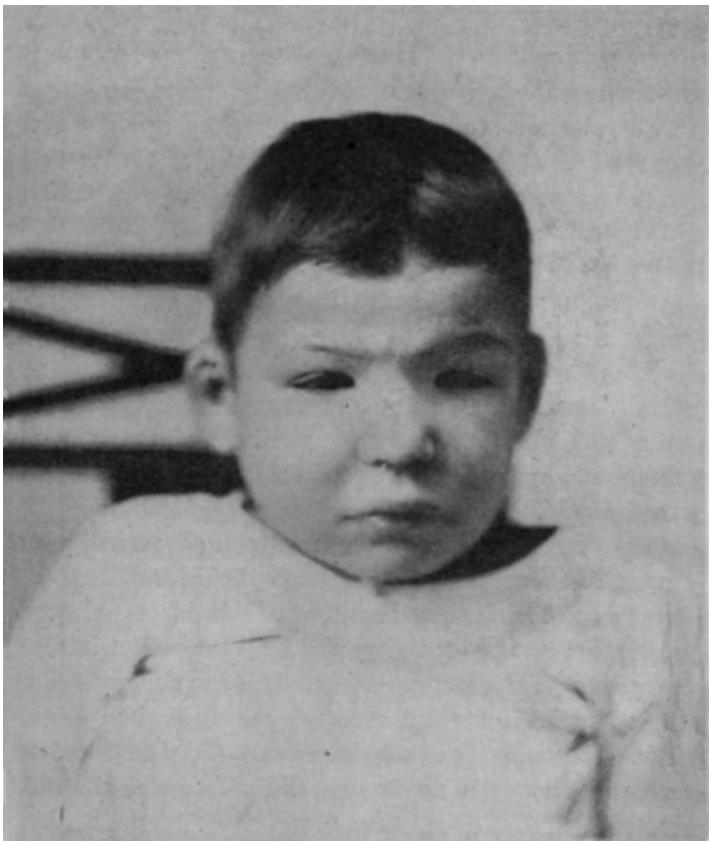

FIG. 1.

At intervals of four or five weeks there was added to the above picture the signs of an acute erysipelas. This began at the side of the nose and extended more or less widely. With such recurrences the temperature, otherwise normal, became elevated, and the patient became generally ill. These recurrences varied in severity, some

being mild, and one being as severe as that related early in the history when the patient was in the Cook County Hospital. This second very severe attack occurred in the first part of December, 1907, and was associated with unilateral otitis media. From the discharge from the ear blood-agar plates were prepared in which only hemolyzing colonies of streptococci developed. The culture of streptococcus obtained from the aural discharge was used for the later injections.

January 17, 1908: In the absence of acute symptoms, the patient was given subcutaneously in the well thigh 500,000,000 streptococci killed with galactose solution. The accompanying chart gives the course of the index after the injections (Chart 9). No local reaction followed the injection, except a slight deep induration, which was not tender to touch and disappeared after six days.

January 22: There was a mild recurrence with limited redness of the face and increased discharge from the sinus of the thigh.

January 24: The patient was given 250,000,000 streptococci killed with galactose solution, subcutaneously. Very slight deep induration followed the injection as before.

January 31: The face was more swollen and the skin was red about the nose. 250,000,000 streptococci killed with galactose solution were injected.

February 1: A moderately severe recurrence occurred, associated with profuse discharge from the sinuses in the thigh.

February 6: The patient had returned to his usual condition.

February 11: 500,000,000 streptococci killed with galactose solution were given. For about a week he seemed to do very well and on February 18 a mild recurrence occurred, mostly limited to one side of the face. From this time on he improved rapidly and continuously.

March 16: There was no swelling of the face with the exception of slight thickening of the upper lip and slight edema of the lower eyelids. Temperature normal. Sinus almost closed. Patient looks and feels well.

March 30: Boy seemed very well. One sinus in thigh discharged a little. There was no swelling of the face. The tissues were soft and free from edema. The nostrils were wide open, and there was slight nasal discharge.

April 21: Was very well. Had gained in weight and general con-

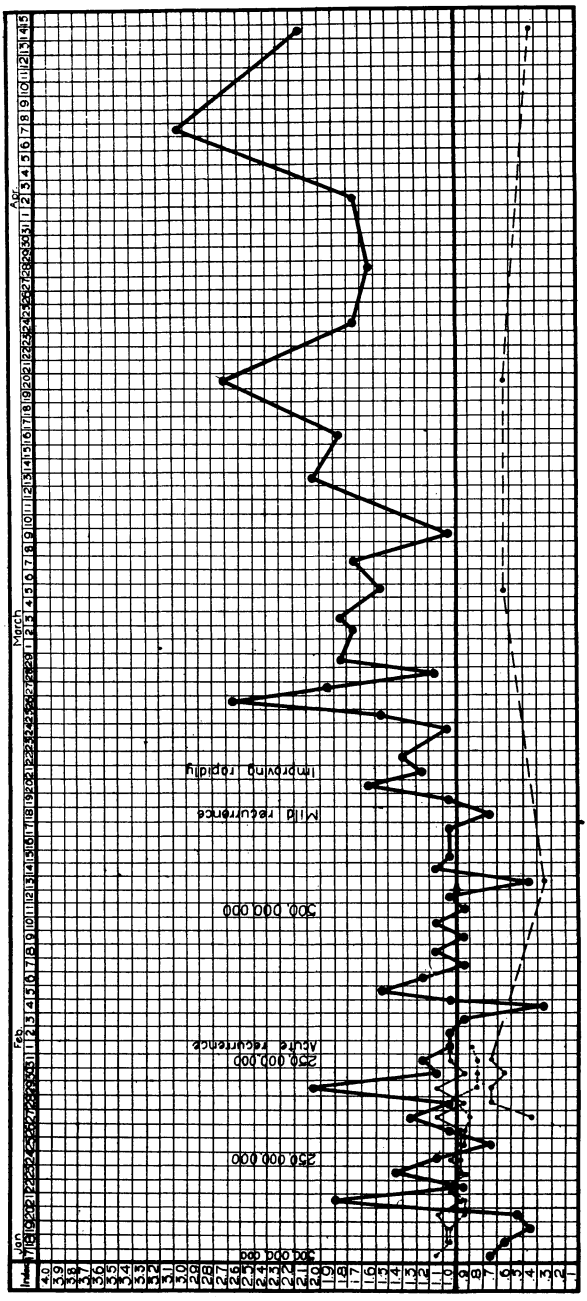

CHART 9.—Opsonic indices in Case II, p. 599; heavy solid line for streptococcus, lighter solid line for *Staphylococcus albus*; heavier broken line for tubercle bacillus, lighter broken line for pneumococcus. The figures represent the approximate number of streptococci injected.

dition was much improved. No swelling about the eyes. Conjunctivae appeared normal. Nostrils wide open. Temperature normal. The bridge of the nose and the upper lip are still slightly thicker than normal. One sinus in the thigh discharged a few drops daily. No streptococci in cultures from this discharge. The patient's father now insisted on taking the boy from the hospital and for some time he was lost track of. October 1 he was found by a visiting nurse and she reported that he had had no trouble in the interval and that his face looked perfectly normal.

In this case no pronounced improvement occurred until about a month after the first injection. During this period the opsonic index fluctuated from a little above to a little below normal. From the time the improvement in the condition of the face began and was progressive, the oposonic index remained constantly above normal. Each injection caused a fall in the streptococco opsonic index, followed by a rise. After each injection the leucocytes increased, corresponding to the fall in the index; with the rise in the index the leucocytes decreased.

Following the first two injections the indices were estimated daily with respect to staphylococcus (*albus*) and the pneumococcus as well as the streptococcus. As shown in the chart with a varying streptococco-opsonic index, the index for staphylococcus and pneumococcus constantly remained practically normal. The tuberculo-opsonic index was found below normal whenever estimated, varying from 0.4 to 0.7.

From these observations it would appear that the opsonic index for the streptococcus rose with the formation of anti-streptococcus bodies, and that the constantly elevated index corresponded to a considerable degree of immunity. The rise and fall of the index following the injections indicated the reaction of the body to the substances introduced; but only when the index stayed permanently above normal did the clinical picture indicate that the body was able to cope with the long infecting streptococci.

In each of the cases just described the streptococcus infection had been present for a considerable time, and the reactions of the body seemed unable to rid it of the infecting organisms. In Case II after each acute attack of erysipelas there was a stage of relative immunity,

but it never reached a degree sufficient to overcome the chronic infection in the tissues in and about the nose, and as this relative immunity wore off an acute attack again recurred.

These cases seem so convincing as to the therapeutic value of the injections of homologous streptococci killed by chemically indifferent agents that such injections are recommended in all cases of chronic streptococcus infection. It must be remembered that marked results may not occur until several injections have been administered, and a temporary aggravation of the clinical signs may follow an injection for a short time. The improvement appears only after a considerable degree of immunity has been established. The only available means of measuring immunity against the streptococcus is the estimation of the opsonin in the serum. The observations here recorded seem to indicate that the amount of opsonin in the blood serum is a valuable index of the degree of immunity, although of course other immune bodies may also be present.

In view of the results obtained in efforts to protect rabbits against virulent streptococci by injections of streptococci killed by heat, it is doubtful if it is of any advantage to inject patients with streptococci killed in this manner.

It may not be amiss to insist upon the necessity of the study of each pathogenic bacterium after it has been killed in various ways to determine the effects of its injection in suitable animals as to the production of immune bodies. This should in every case precede the use of the so-called "vaccine" in man. No general conclusions can safely be drawn from the study of one variety of bacterium as to the properties and activities of others.

Owing to difficulty in securing rabbits, no study has been made of the protective action of injections of galactose killed streptococci of one strain against other strains. In the absence of observations on this point the use of heterologous streptococcal "vaccines" cannot be said to have any good experimental basis.

Gabritschewsky[1] and several other observers, mostly Russian, have employed injections of killed streptococcus cultures to produce immunity against scarlatina. Gabritschewsky used broth cultures, heated to 60° C. and preserved with 0.5 per cent phenol. Following

[1] *Centralbl. f. Bakt.*, Orig., 1906, 41, pp. 719, 844; *Berl. klin. Wchnschr.*, 1907, 44, p. 556.

the injection of such material they have observed an erythematous eruption, a slight rise in temperature, angina, and sometimes vomiting. These symptoms do not appear in children who have passed through scarlatina, and after children have been subjected to these injections they do not develop scarlatina even though not isolated from those having clinical scarlet fever. With a great variability in susceptibility to scarlatina among children and with great difficulty in adequately controlling observations, it is not surprising that much skepticism should exist regarding the value to be attached to these observations.

It is desirable, however, that these observations should be repeated under circumstances where proper control as to exposure, etc., is possible. In such cases it may be suggested that streptococci isolated from cases of acute scarlatina be injected after being killed by galactose or other chemically indifferent agents, since this can be done without apparent injury to the person injected.

CONCLUSIONS.

Injections into rabbits of streptococci killed by suspension in 25 per cent galactose solution gives rise to a greater or less degree of protection against the subsequent injection of homologous living virulent organisms.

Such injections are followed by a marked increase in streptococco-opsonin, and a persistently high index may be taken to indicate a condition of immunity.

Whether the amount of opsonin is a measure of the full degree of immunity cannot be stated.

Injections into rabbits of streptococci killed by heat do not protect them against the subsequent injection of homologous living virulent organisms, but may even lower their natural resistance.

Such injections are not followed by any considerable increase in streptococco-opsonin.

The results of the injection in rabbits of streptococci killed by heat may throw some light upon the questionable results obtained by the injection of heat-killed streptococci in man in cases of streptococcal infection.

In cases of subacute and chronic infection by streptococci in man, therapeutic injections of homologous streptococci killed by chemically

indifferent agents are recommended. The use of streptococci killed by heat in such cases is of at least doubtful benefit.

In the case of every pathogenic bacterium the immunizing effect of the bacteria killed in various ways should be studied in animals and injections of such materials were better employed in man only when substantial favorable results have been observed in animals.

Until killed heterologous streptococci are shown to be active against various strains of streptococci, injections of homologous streptococci are preferable.

It is desirable to study the injection in children of streptococci isolated from cases of scarlet fever and killed by chemically indifferent agents with respect to its protective effects against scarlet fever.